MOLECULAR BACKGROUND AND GENOTYPE-PHENOTYPE CORRELATION IN APECED PATIENTS FROM CAMPANIA AND IN THEIR RELATIVES

MOLECULAR BACKGROUND AND GENOTYPE-PHENOTYPE CORRELATION IN APECED PATIENTS FROM CAMPANIA AND IN THEIR RELATIVES.

D. Capalbo[1], C. Mazza[2], R. Giordano[3], N. Improda[1], E. Arvat[4], S. Cervato[5], L. Morlin[5], C. Pignata[1], C. Betterle[5] and M. Salerno[1]

[1]Department of Pediatrics, University "Federico II" of Naples; [2]Institute for Molecular Medicine A. Nocivelli, University of Brescia; [3]Department of Clinical and Biological Sciences and [4]Division of Endocrinology , Department of Internal Medicine, University of Turin; [5]Division of Endocrinology, Department of Medical and Surgical Sciences, University of Padua, Italy

Short title: Genotype in APECED patients from Campania

Key Words: APECED, AIRE, genotype-phenotype correlation, autoimmune diseases, pediatric endocrinology

Correspondence and reprints requests to

Mariacarolina Salerno, MD, PhD, Department of Pediatrics, "Federico II" University of Naples, Italy, Tel +390817464339, FAX +390815451278, Email: salerno@unina.it

Acknowledgements: this study was supported in part by the EU Research Project EurAPS: Autoimmune polyendocrine syndrome type I–a rare disorder of childhood as a model for autoimmunity, contract number LSHM-CT 2005-005223, and in part by a grant from European Union Seventh Framework Programme, the Euradrenal project: Pathophysiology and Natural Course of Autoimmune Adrenal Failure in Europe. Grant Agreement No. 2008-201167.

Word counts: 2479

Number of references: 26

Number of tables: 1

Number of figures: 1

Disclosure summary: the authors have nothing to declare.

LIST OF ABBREVIATIONS

Addison's disease: AD

Autoimmune Hepatitis: AH

Atrophic Gastritis: AG

Autoimmune Thyroiditis: AT

AutoImmune REgulator gene: AIRE

Autoimmune Polyendocrinopathy - Candidiasis – Ectodermal - Distrophy: APECED

Autoantibodies: Abs

Autoantibodies against Adrenal Cortex: ACA

Autoantibodies against Aromatic-L-Aminoacid decarboxylase: AADCAbs

Autoantibodies against Glutamic acid decarboxylase: GADA

Autoantibodies against Islet cells: ICA

Autoantibodies against Intrinsic Factor: IFA

Autoantibodies against 17α-hydroxilase: 17α-OHAbs

Autoantibodies against 21 hydroxilase: 21-OHAbs

Autoantibodies against melanin producing cells: MPCA

Autoantibodies against Parietal cells: PCA

Autoantibodies against Side-chain cleavage enzyme: sccAbs

Autoantibodies against Steroid-producing cells: StCA

Autoantibodies against tissue transglutaminase: tTgAbs

Autoantibodies against Thyroglobulin: TgAbs

Autoantibodies against Thyroid microsomal: TMAbs

Autoantibodieas against Tryptophan hydroxylase: TPHAbs

Autoantibodies against Thyroperoxidase: TPOAbs

Caspase-recruitment domain: CARD

Chronic Hypoparathyroidism: CH

Chronic Mucocutaneous Candidiasis: CMC

Ectodermal Dystrophy: ED

Growth Hormone Deficiency: GHD

Hypergonadotropic Hypogonadism: HH

Posterior Reversible Encephalopathy Syndrome: PRES

ABSTRACT

Background: Autoimmune-Polyendocrinopathy-Candidiasis-Ectodermal-Distrophy (APECED) is a recessive disease, caused by mutations in the AutoImmune REgulator (AIRE) gene. Different mutations are peculiar of particular populations. In Italy three hot spots areas where APECED shows an increased prevalence, have been identified in Sardinia, Apulia and in the Venetian region.

Aim: in this study we analyzed AIRE mutations and genotype-phenotype correlation in APECED patients originating from Campania and in their relatives.

Patients and methods: in six patients affected with APECED clinical findings, genetic analysis of AIRE and APECED-related autoantibodies were performed.

Results: all patients carried at least one mutation on exon 1 or on splice-site flanking exon 1. Two siblings carried a complex homozygous mutation [IVS1 + 1G>C; IVS1 + 5delG] on intron 1; two patients were compound heterozygous for [T16M]+[W78R] (exons 1+2); one patient was compound heterozygous for [A21V]+[C322fs] (exons 1+8) and another was homozygous for [T16M]+[T16M] on exon 1. Expression of the disease showed wide variability while circulating autoantibodies paralleled to phenotype in each patient. Analysis of relatives allowed the identification of 8 heterozygotes. None of heterozygous subjects presented major findings of APECED.

Conclusions: mutations localized on exon 1 and the region flanking exon 1 are common in APECED patients originating from Campania. Genotype-phenotype correlation failed to reveal a relationship between detected mutations and clinical expression. Mutations in heterozygosis in AIRE gene are not associated to major findings of APECED.

INTRODUCTION

Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal-Distrophy (APECED) is a rare autosomal recessive disease (OMIM 240300) which affects many tissues especially endocrine glands (1). The diagnosis is primarily based on the presence of two out of the three most common clinical features: chronic mucocutaneous candidiasis (CMC), chronic hypoparathyroidism (CH) and Addison's disease (AD). CMC is often the first clinical manifestation to appear before the age of 5 year, followed by CH and later by AD. APECED is caused by mutations in the AutoImmune REgulator gene (AIRE), which maps to chromosome 21q22.3 (2, 3) and encodes a 55-kDa protein that acts as a transcription regulator. Over 60 mutations have by now been localized in the AIRE genes of different APECED patients (4). Even though it occurs through the world, its incidence is higher in some genetically isolated populations. The estimated prevalence of APECED is 1:9.000 in Iranian Jewes (5), 1:25.000 in Finns (6,7) and 1:14.400 in Sardinians (8). Some different mutations have been found to be peculiar of specific areas. R257X is the most common mutation among Finnish and other European patients (9-11), 1094-1106del113 (or 967-979del13 bp) is the most common mutation in British (12), Irish (13), North America (14,15) and Norwegian patients (16) and the Y85C mutation is more frequent among Iranian Jewes (17).

In Italy three hot spots areas where APECED shows an increased prevalence, have been identified in Sardinia, Apulia and in the Venetian region. Moreover, both in Sardinia and Apulia a peculiar mutation of AIRE has been identified: the mutation R139X on exon 3 in Sardinia (18) and the mutation W78R on exon 2 in Apulia (19). In Veneto region AIRE gene mutations were different from the other Italian regions but similar to that identified in finnish and anglo-saxon patients (20). A typical mutation has been recently identified also in Sicily (R203X on exon 5) (20).

However, the different mutations have not to date been convincingly associated with particular disease manifestations (4).

To the best of our knowledge, only one patient with AIRE mutation was described from Campania so far, having the homozygous mutation c1314-1326 del13/insGT on exon 11 (21).

Aim of this study was to characterized genotype and phenotype of 6 new APECED patients originating from Campania, in the attempt to evaluate their genotype-phenotype correlation. Moreover we studied the relatives of these patients in order to evaluate the clinical and biochemical effects of heterozygous mutations of the gene.

PATIENTS AND METHODS

Patients

Six patients originating from Campania (a region of Southern Italy) affected with APECED (5 F, 1 M), were investigated. In all of them the onset of the disease was in early childhood; two of them were followed up until young adult age. Patients were selected for the presence of signs or symptoms suggestive of APECED. Two of the three major criteria of APECED (hypoparathyroidism and chronic candidiasis) were present at diagnosis in 5 of them. One patient presented only candidiasis as major criteria, but she was enrolled for the association of chronic mucocoutaneous candidiasis with other autoimmune diseases (vasculitis, autoimmune thyroiditis, alopecia) in early childhood. The patients were originating from 5 unrelated families. They were all originating from Campania region, but the areas where they lived or from they had their origins were not close to each other. Consanguinity between the parents was identified in only one family with two affected children (third cousins). In the 6 patients a complete clinical and biochemical evaluation of the major and minor signs and symptoms of APECED disease, molecular analysis of AIRE gene and assessment of all APECED-related autoantibodies were performed.

Major and minor clinical features of APECED, molecular analysis of AIRE and autoantibodies related to APECED syndrome were also evaluated in eight out of the ten parents of the affected patients (4M, 4F) (aged 40±10 years), who gave their consent. They all were originating from Campania.

Analysis of AIRE gene

Genomic DNA was extracted from peripheral blood by Maxwell 16 Systems (Promega Corporation, Madison WI, USA). All 14 exons of the AIRE gene were amplified with the use of primers located

on the respective flanking introns (17) and were analyzed by direct sequencing using the Big Dye terminator cycle sequencing Kit and ABI PRISM 3130 automated sequencer (Applied Biosystems, Foster City, CA, USA). The analysis included sequencing of the donor/acceptor sites of all the introns.

Autoantibodies

Autoantibodies against the following antigens were performed by classical indirect immunofluorescence technique, complement fixation, ELISA or RIA, as appropriate: Thyroglobulin (TgAbs), Thyroid microsomal (TMAbs), Thyroperoxidase (TPOAbs), Parietal cells (PCA), Intrinsic factor (IFA), Islet cells (ICA), Glutamic acid decarboxylase (GADA), tissue transglutaminase (tTgAbs), Adrenal cortex (ACA), Steroid-producing cells (StCA), melanin producing cells (MPCA). Autoantibodies to 21-hydroxilase (21-OHAbs) were tested using RSR's kit (22). 17α-hydroxilase (17α-OHAbs) as well as side-chain cleavage enzyme (sccAbs) autoantibodies were measured using specific and sensitive immunoprecipitation assays (IPAs) (23). Autoantibodies to Aromatic-L-Aminoacid decarboxylase (AADCAbs) and Tryptophan hydroxylase (TPHAbs) were measured in an immunoprecipitation assay using (35)S-labelled full length and fragments of TPH and AADC (24).

RESULTS

Clinical presentation and autoantibodies

Clinical characteristics and autoantibodies' profile of each patient are reported in Table 1.

Patient 1 was born from consanguineous parents (third cousins). He first presented at 1.5 years of age with a history of transient hypertransaminasemia. At the age of 5 years, he suddenly developed an unusually severe phenotype with many signs and symptoms outbreaking over a six month period. In fact, the physical examination revealed the presence of vitiligo, nail dystrophy, alopecia, oral candidiasis and hepatosplenomegaly. He also suffered from alternating stipsis/diarrhea and chronic abdominal pain. Laboratory testing led to diagnosis of autoimmune hepatitis, chronic hypoparathyroidism, chronic thyroiditis and Addison's disease. Endoscopy revealed the presence of atrophic gastritis of the body. Six months after the onset of this accelerated phase, the patient

suddenly developed a severe neurological syndrome with neuroradiological findings suggestive of Posterior Reversible Encephalopathy Syndrome (PRES), a life-threatening event never described before in APECED patients (**Table 1**) (25). Screening of autoantibodies revealed the presence of TgAbs, TMAbs, TPOAbs, PCA, ACA, 21-OH-Abs, StCA, 17α-OHAbs, AADCAbs, TPHAbs and MPCA. sccAbs and GADA were negative (**Table 1**).

Patient 2 (the younger sister of patient 1), presented at 4 years of age with chronic hypoparathyroidism, chronic mucocoutaneous candidiasis and ectodermal dystrophy (**Table 1**). During three years of follow-up, she did not develop any other feature of the disease. None of the autoantibodies tested resulted positive (**Table 1**).

Patient 3 first presented at the age of 2 years with chronic mucocoutaneous candidiasis, chronic abdominal pain and alternating stipsis/diarrhea. At the age of 8 years she was diagnosed as having chronic hypoparathyroidism, Addison's disease, growth hormone deficiency, enamel dysplasia and alopecia. At the age of 14 years she developed ungueal dystrophy, chronic thyroiditis and hypergonadotropic hypogonadism (**Table 1**). Autoantibodies' profile revealed the presence of TgAbs, TMAbs, TPOAbs, ICA, ACA, 21-OH-Abs, StCA, 17α-OHAbs, sccAbs, AADCAbs and TPHAbs (**Table 1**).

Patient 4 first presented at the age of 8 months with vasculitis and splenomegaly. Thereafter, at the age of 2 years, she developed chronic mucocutaneous candidiasis and, at the age of 5 years, severe alopecia, chronic thyroiditis and recurrent abdominal pain with alternating stipsis/diarrhea (**Table 1**). Autoantibodies' profile revealed the presence of TgAbs, TMAbs, PCA, ACA, 21-OH-Abs StCA, 17α-OHAbs and sccAbs (**Table 1**).

Patient 5 presented with chronic hypoparathyroidism at the age of 2 years. At the age of 3 years she developed Addison's disease, chronic oral candidiasis and ungueal dystrophy (**Table 1**). Only 21-OHAbs resulted to be positive. sccAbs, AADCAbs and TPHAbs were negative. During 4 years of follow-up, she did not show any other signs, symptoms or autoantibody (**Table 1**).

Patient 6 first developed chronic hypoparathyroidism, enamel dysplasia and chronic mucocoutaneous candidiasis at the age of 6 years. At the age of 15 years, Addison's disease and

alopecia were also diagnosed (**Table 1**). Autoantibodies' profile revealed positivity for ACA, 21-OHAbs, and sccAbs (**Table 1).**

Noteworthy, autoantibodies paralleled clinical phenotype in each case. Infact, no antibodies were detected in patient 2 with only a mild expression of the disease whereas several autoantibodies were detected in patients 1 and 3 with a more severe expression of APECED. Globally, the most prevalent autoantibodies found were those against 21-Hydroxilase.

AIRE mutation analysis

Five different AIRE mutations were detected. Interestingly, all patients carried at least one mutation on exon 1 or on splice donor site of intron 1 (**Table 1**).

Two siblings carried a complex homozygous mutation in intron 1, consisting of a substitution of IVS1 + 1G by C accompanied in *cis* by a single nucleotide deletion at IVS1 + 5G residue [IVS1 + 1G>C; IVS1 + 5delG]. Two patients were compound heterozygous for [T16M]+[W78R] on exons 1 and 2 respectively; one patient was compound heterozygous for [A21V]+[C322fs] on exons 1 and 8, respectively and one patient was homozygous for [T16M] +[T16M] on exon 1. None of the mutation described is a novel mutation but the complex variant [IVS1 + 1G>C; IVS1 + 5delG] is uncommon. Figure 1 summarizes on both the gene and protein domains mutations detected in this study, as well as those previously described.

Relatives

Analysis of AIRE gene revealed that all parents were heterozygotes for one of the mutations found in the patients, confirming autosomal recessive model of inheritance of APECED. Three of them had a mutation on exon 1, two had a complex mutation in intron 1, two had a mutation on exon 2 and one had a mutated allele on exon 8.

Clinical evaluation revealed that none of them was affected by one of the major features of APECED. Four of them (3F, 1M) had positive autoantibodies: two against PC, one against TM and another against both Tg and PC.

DISCUSSION

Recent studies have documented that in four italian regions different AIRE gene mutations have been identified. Typical mutations have been identified in Sardinia (R139X on exon 3) (18) , in Apulia (W78R on exon 2) (19), in Veneto (R257X on exon 6 and 8) and in Sicily (R203X on exon 5) (20).

In Campania, a region of the Southern Italy, only one case with AIRE mutation has been described so far, carrying an homozygous mutation on exon 11 (c1314-1326 del 13/insGT) (21).

In our study we have delineated the molecular pathology and the clinical spectrum of 6 more probands affected with APECED originating from this region.

Our results suggest that mutations localized on exon 1 or on the region flanking exon 1, being present either in compound heterozygosis or homozygosis, are relatively common in APECED patients originating from Campania.

Exon 1 and the region flanking exon 1, are localized at the aminoterminus of AIRE. This domain was long referred to as a homogeneously staining region (HSR) domain but recent evidence suggest that this region encompasses a caspase-recruitment domain (CARD) (26). As shown in figure 1, in this area most other missense mutations have been located. CARD domains are involved in the process of homo- or hetero-dimerization. Aire's CARD is needed for a correct dimerization of AIRE and interactions with other transcriptional control proteins. Thus, missense mutations and also small deletions affecting this domain lead to the production of a functionally defective protein due to the loss of its homodimerization properties (26).

None of the mutations detected in our patients is novel. However the complex homozygous mutation reported in the two siblings is uncommon. The sequence variant [IVS1 + 1G>C; IVS1 + 5delG] consists of two mutations, each likely to affect the splicing of intron 1. The IVS1 + 1G is 100% conserved in the major-class introns so that its change into C must render the splice site nonfunctional. The IVS1 + 5delG is also likely to have a negative effect because in the major-class introns at the IVS+5 position only G,A or T occur. This mutation has never been reported in APECED patients from Italy. So far, the IVS1 + 1G>C; IVS1 + 5delG mutation has been described in heterozygous state with the R257X in a single individual from Poland (10).

Noteworthy, in the six patients described the phenotype was characterized by the presence of several unusual (GH deficiency, vasculitis) and life-threatening (PRES, Chronic Hepatitis) complications of the disease. In particular, the PRES is a neurological acute syndrome never described before in APECED patients (25). GH deficiency and vasculitis also represent very rarely a feature of the disease. Moreover, three of our six patients, presented Addison's disease in early childhood, whereas it is commonly reported after the second decade of life. In patients with early development of Addison, life-threatening complications (such as autoimmune hepatitis and squamous cell carcinoma of the oral mucosa) can occur, and therefore they should be followed more closely.

Analysis of genotype-phenotype correlation in our subjects failed to reveal a clear relationship, as previously reported in other series of patients. All patients had an early onset of the disease, but the phenotype and the severity of the disease widely differed even between patients with the same mutation of AIRE. In particular the 2 siblings carrying the same complex homozygous mutation (patient 1 and 2) showed a wide heterogeneity of clinical expression: one patient developed a severe phenotype culminating in a life-threatening event,whereas his sister presented with only a mild phenotype. Moreover, their phenotypes were different from the only other patient from Poland carrying the same complex mutation. Patient 3 and 4, presenting the same missense mutations [T16M]+[W78R], also widely differed in their phenotype: the first patient, in fact, had only a mild phenotype characterized by alopecia, ectodermal dystrophy and candidiasis, whereas the second patient developed a severe phenotype, with many signs and symptoms, since the first decade of life. Also in patients with Addison, the early development of disease was not apparently related to the genotype in that the three patients affected carried different mutations of the gene.

Autoantibodies paralleled clinical phenotype in each case, confirming their role in the pathogenesis of the disease.

The study of the relatives confirmed that the heterozygous state for AIRE mutations is not associated to the presence of major signs or symptoms correlated to APECED syndrome, as previously reported (20). Accordingly with Cervato et al, in fact, in our heterozygous subjects we only detected latent autoimmune diseases as demonstrated by the presence of thyroid or PC autoantibodies in 4 of the 8 studied heterozygous. We found only a slight prevalence in female versus male, whereas no

differences were detected on the basis of age. However, in all relatives, also those initially negative, a longitudinal evaluation of antibodies could be useful in order to identify those subjects that might develop clinical or subclinical disease over time.

In conclusion we described 6 patients characterized by several unusual and life-threating complications of APECED. Our analysis failed to reveal a clear genotype-phenotype correlation according to previous reports. However, our data demonstrate that APECED in Campania region is more frequent than reported so far and that mutations on exon 1and on region flanking exon 1 of AIRE gene are common in patients originating from this region.

References

1. Betterle C, Greggio NA & Volpato M. Clinical Review 93: Autoimmune polyglandular syndrome type 1. J Clin Endocrinol Metab 1998, 83: 1049-1055.
2. Finish-German APECED Consortium. An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains. Nat Genet, 17: 399-403.
3. Nagamine K, Peterson P, Scott HS, et al. Positional cloning of the APECED gene. Nat Genet 1997, 17: 393-398.
4. Mathis D & Benoist C Aire. Annu Rev Immunol 2009, 27: 287-312.
5. Zlotogora J & Shapiro MS. Polyglandular autoimmune syndrome type I among Iranian Jewes. J Med Genet 1992, 29: 824-826.
6. Aaltonen J, Bjorses P, Sandkuijl L, Perheentupa J, Peltonen L. An autosomal locus causing autoimmune disease: autoimmune polyglandular disease type I assigned to chromosome 21. Nat Genet 1994, 8: 83-87.
7. Ahonen P, Myllarniemi S, Sipila I, Perheentupa J. Clinical variation of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) in a series of 68 patients. N Engl J Med 1990, 322: 1829-1836.

8. Rosatelli MC, Meloni A, Devoto M et al. A common mutation in Sardinian autoimmune-polyendocrinopathy-candidiasis-ectodermal dystrophy patients. Hum Genet 1998, 103: 428-434.

9. Podkrajsek KT, Bratanic N, Krzisnik C, Battelino T. Autoimmune regulator-1 messenger ribonucleic acid analysis in a novel intronic mutation in a cohort of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy patients. J Clin Endocrinol Metab 2005, 90: 4930-4935.

10. Stolarski B, Pronicka E, Korniszewski L et al. Molecular background of polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome in a Polish population: novel AIRE mutations and an estimate of disease prevalence. Clin Genet 2006, 70: 348-354.

11. Scott HS, Heino M, Peterson P et al. Common mutations in autoimmune polyendocrinopathy-candidiasis-ectodermal-dystrophy patients of different origins. Mol Endocrinol 1998, 12: 1112-1119.

12. Pearce SH, Cheetham T, Imrie H et al. A common and recurrent 13-bp deletion in the autoimmune regulator gene in British kindreds with autoimmune polyendocrinopathy Type 1. Am J Hum Genet 1998, 63: 1675-1684.

13. Dominguez M, Crushell E, Ilmarinen T et al. Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) in the Irish population. J Pediatr Endocrinol Metab 2006, 19: 1343-1352.

14. Wang CY, Davoodi-Semiromi A, Huang W, Connor E, Shi JD, She JX. Characterization of mutations in patients with autoimmune Polyglandular syndrome type 1 (APS 1). Hum Genet 1998, 103: 681-685.

15. Heino M, Scott HS, Chen Q et al. Mutation analyses of North American APS-1 patients. Hum Mutat 1999, 13: 69-74.

16. Wolff ASB, Erichsen MM, Meager A et al. Autoimmune polyendocrine syndrome type 1 (APS I) in Norway-phenotipic variation, autoantibodies and novel mutations in the autoimmune regulator (AIRE) gene. J Clin Endocrinol Metab 2007, 92: 595-603.

17. Bjorses P. Halonen M, Palvimo JJ et al. Mutations in the AIRE gene: effects on subcellular location and transactivation function of the autoimmune polynedocrinopathy-candidiasis-ectodermal dystrophy protein. Am J Hum Genet 2000, 66: 378-392.

18. Clemente MG, Meloni A, Obermyer-Straub P, Frau F, Manns MP, De Virgiliis S. Two cytochrome P450 are major hepatocellular autoantignes in autoimmune polyglandular syndrome type 1. Gastroenterology 1998, 114: 324-328.

19. Meloni A, Perniola R, Faà V, Corvaglia E, Cao A, Rosatelli MC. Delineation of the molecular defects in the AIRE gene in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy patients from Southern Italy. J Clin Endocrinol Metab 2002, 87: 841-846.

20. Cervato S, Mariniello B, Lazzarotto F et al. Evaluation of the autoimmune regulator (AIRE) gene mutations in a cohort of Italian patients with autoimmune-polyendocrinopathy-candidiasis-ectodermal-dystrophy (APECED) and in their relatives. Clin Endocrinol 2009, 70: 421-428.

21. Lintas C, Cappa M, Comparcola D, Nobili V, Fierabbracci A. An 8-year-boy with autoimmune hepatitis and Candida onychosis as the first symptoms of autoimmune polyglandular syndrome (APS 1): identification of a new homozygous mutation in the autoimmune regulator gene (AIRE). Eur J Pediatr 2008, 167: 949-953.

22. Tanaka H, Perez MS, Powell M et al. Steroid 21-Hydroxylase Autonatibodies: Measurements with a New Immunoprecipitation Assay. J Clin Endocrinol Metab 1997, 82: 1440-1446.

23. Chen S, Sawicka J, Betterle C et al. Autoantibodies to steroidogenic enzymes in autoimmune polyglandular syndrome, Addison's disease, and premature ovarian failure. J Clin Endocrinol Metab 1996, 81: 1871-1876.

24. Dal Pra C, Chen S, Betterle C et al. Autoantibodies to human tryptophan hydroxylase and aromatic L-aminoacid decarboxylase. Eur J Endocrinol 2004, 150*:* 313-321.

25. Capalbo D, Elefante A, Spagnuolo MI et al. Posterior reversible encephalopathy syndrome during an accelerated phase of a severe APECED phenotype due to an uncommon mutation of AIRE. Clin Endocrinol 2008 , 69**:** 511-513.

26. Ferguson BJ, Alexander C, Rossi SW et al. AIRE's CARD revealed, a new structure for central tolerance provokes transcriptional plasticity. J Biol Chem 2008, 283: 1723-1731.

Table 1: Clinical manifestations, autoantibodies and AIRE mutations in the 6 APECED patients from Campania

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Sex	M	F	F	F	F	F
Age at the onset (years)	1.5	4.0	2.0	0.7	2.0	6.0
Symptoms/Signs	CMC CH AD AT AG ED AH Stipsis/diarrhea Alopecia Vitiligo PRES	CMC CH ED	CMC CH AD HH AT ED Enamel dysplasia Stipsis/diarrhea Alopecia GHD	CMC AT Stipsis/diarrhea Alopecia Vasculitis	CMC CH AD ED	CMC CH AD Enamel dysplasia Alopecia

Positive Autoantibodies	TgAbs TMAbs TPOAbs PCA ACA 21OHAbs StCA 17-α-OHAbs AADCAbs TPHAbs MPCA		TgAbs TMAbs TPOAbs ICA ACA 21-OHAbs StCA 17-α-OHAbs sccAbs AADCAbs TPHAbs	TgAbs TMAbs PCA ACA 21-OHAbs StCA 17-α-OHAbs sccAbs	21-OHAbs	ACA 21-OHAbs sccAbs
Mutation	IVS1+1G>C;IVS1+5delG	IVS1+1G>C;IVS1+5delG	T16M/W78R	T16M/W78R	A21V/C322fs	T16M/T16M
Exons	1/1	1/1	1/2	1/2	1/8	1/1

AD, Addison's disease; AG, Atrophic Gastritis; AH, Autoimmune Hepatitis; AT, Autoimmune Thyroiditis; CH, Chronic Hypoparathyroidism; CMC, Chronic Mucocutaneous Candidiasis; ED, Ectodermal Dystrophy; HH, Hypergonadotrpoic Hypogonadism; GHD, Growth Hormone Deficiency; PRES, Posterior Reversible Encephalopathy Syndrome.

AADCAbs, autoantibodies against Aromatic L-Amino Acid Decarboxylase; ACA, autoantibodies against Adrenal Cortex; GADA, autoantibodies against Glutamic Acid Decarboxylase; 17α-OHAbs, autoantibodies against 17α-Hydroxylase; 21-OHAbs, autoantibodies against 21-Hydroxylase; ICA, autoantibodies against Islet Cells; MPCA, autoantibodies against Melaning Producing Cells; PCA, autoantibodies against Parietal Cells; sccAbs, autoantibodies against side chain cleavage enzyme; StCA, autoantibodies against Steroid Producing Cells; TgAbs, autoantibodies against Thyroglobulin; TMAbs, autoantibodies against Thyroid Mycrosomal; TPOAbs, autoantibodies against Thyroperoxidase; TPHAbs, autoantibodies against Tryptophan Hydroxilase.

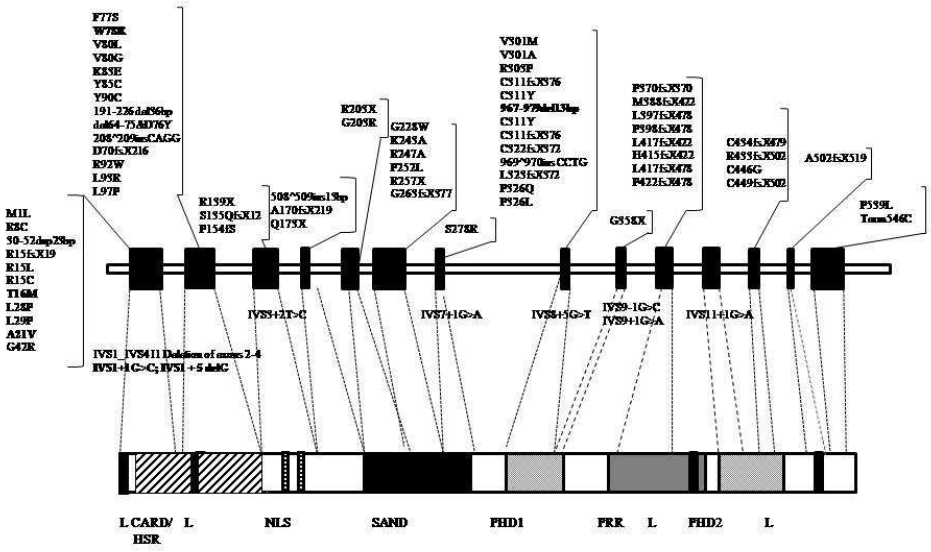

Figure 1. AIRE gene (top) and corresponding protein (bottom) with functional domains. Mutations detected so far are listed. Mutations found in our patients are in bold.

254x190mm (96 x 96 DPI)